

**Amendment and Response**

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Confirmation No.: 6204

Filed: 21 March 2001

For: PRIMERS FOR USE IN DETECTING BETA-LACTAMASES

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Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

12. (Previously presented) A primer selected from the group of:  
5' - CGT CGC TCA CCA TAT CTC CC - 3' (SEQ ID NO:34);  
5' - CCT CTC GTG CTT TAG ACC CG - 3' (SEQ ID NO:35); and full-length complements thereof.
13. (Previously presented) A primer selected from the group of:  
5' - CGC TGG GAA ACC TAT TCG G - 3' (SEQ ID NO:36);  
5' - CTG CCA TCC AGT TTC TTC GGG - 3' (SEQ ID NO:37); and full-length complements thereof.
14. (Previously presented) A primer selected from the group of:  
5' - GGT GGC ATT GAC AAA TTC TGG - 3' (SEQ ID NO:38);  
5' - CCC ACC ATG CGA CAC CAG - 3' (SEQ ID NO:39); and full-length complements thereof.
15. (Previously presented) A primer selected from the group of:  
5' - TGT GCA ACG CAA ATG GCA C - 3' (SEQ ID NO:40);  
5' - CGA CCC CAA GTT TCC TGT AAG TG - 3' (SEQ ID NO:41); and full-length complements thereof.
16. (Previously presented) A primer selected from the group of:  
5' - AGG CAC GAT AGT TGT GGC AGA C - 3' (SEQ ID NO:42);

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5' - CAC TCA ACC CAT CCT ACC CAC C - 3' (SEQ ID NO:43); and full-length complements thereof.

17. (Currently amended) A method for identifying a beta-lactamase in a clinical sample, the method comprising:

providing a pair of oligonucleotide primers specific for nucleic acid characteristic of the OXA family of beta-lactamase enzymes, wherein the enzyme is found in a Gram-negative bacterium selected from the group of *Enterbacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, *Escherichia coli*, *Providencia spp.*, *Proteus mirabilis*, *Yersinia enterocolitica*, and combinations thereof, excluding OXA-1, ~~10~~, ~~11~~, ~~14~~, ~~16~~, and ~~17~~, wherein one primer of the pair is complementary to at least a portion of the beta-lactamase nucleic acid in the sense strand and the other primer of each pair is complementary to at least a portion of the beta-lactamase nucleic acid in the antisense strand;

annealing the primers to the beta-lactamase nucleic acid;

simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product that is complementary to the nucleic acid strands annealed to each primer wherein each extension product after separation from the beta-lactamase nucleic acid serves as a template for the synthesis of an extension product for the other primer of each pair;

separating the amplified products; and

analyzing the separated amplified products for a region characteristic of [[the]] a beta-lactamase found in a Gram-negative bacterium selected from the group consisting of *Enterbacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, *Escherichia coli*, *Providencia spp.*, *Proteus mirabilis*, *Yersinia enterocolitica*, and combinations thereof.

39. (Original) The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the OXA-9 beta-lactamase enzyme.

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40. (Previously presented) The method of claim 39 wherein the primers are selected from the group of:

5' - CGT CGC TCA CCA TAT CTC CC - 3' (SEQ ID NO:34);

5' - CCT CTC GTG CTT TAG ACC CG - 3' (SEQ ID NO:35); and full-length

complements thereof.

41. (Original) The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the OXA-12 beta-lactamase enzyme.

42. (Previously presented) The method of claim 41 wherein the primers are selected from the group of:

5' - CGC TGG GAA ACC TAT TCG G - 3' (SEQ ID NO:36);

5' - CTG CCA TCC AGT TTC TTC GGG - 3' (SEQ ID NO:37); and full-length

complements thereof.

43. (Currently amended) The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the OXA-5, 6, 7, 10, 11, [[or]] 13, and 14 beta-lactamase enzymes.

44. (Previously presented) The method of claim 43 wherein the primers are selected from the group of:

5' - GGT GGC ATT GAC AAA TTC TGG - 3' (SEQ ID NO:38);

5' - CCC ACC ATG CGA CAC CAG - 3' (SEQ ID NO:39); and full-length complements

thereof.

47. (Original) The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the OXA-2, 3, and 15 beta-lactamase enzymes.

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48. (Previously presented) The method of claim 47 wherein the primers are selected from the group of:

5' - AGG CAC GAT AGT TGT GGC AGA C - 3' (SEQ ID NO:42);

5' - CAC TCA ACC CAT CCT ACC CAC C - 3' (SEQ ID NO:43); and full-length complements thereof.

49. (Currently amended) A diagnostic kit for detecting an OXA family beta-lactamase which comprises packaging, containing, separately packaged:

(a) at least one primer pair capable of hybridizing to beta-lactamase nucleic acid of interest characteristic of an OXA family beta-lactamase, wherein the enzyme is found in a Gram-negative bacterium selected from the group of *Enterbacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, *Escherichia coli*, *Providencia spp.*, *Proteus mirabilis*, *Yersinia enterocolitica*, and combinations thereof, excluding OXA-1, 10, 11, 14, 16, and 17;

(b) a positive and negative control; and

(c) a protocol for identification of the beta-lactamase nucleic acid of interest.

51. (Previously presented) A diagnostic kit for detecting an OXA family beta-lactamase which comprises packaging, containing, separately packaged:

(a) at least one primer pair capable of hybridizing to beta-lactamase nucleic acid of interest;

(b) a positive and negative control; and

(c) a protocol for identification of the beta-lactamase nucleic acid of interest;

wherein the primers are selected from the group consisting

of:

5' - CGT CGC TCA CCA TAT CTC CC - 3' (SEQ ID NO:34);

5' - CCT CTC GTG CTT TAG ACC CG - 3' (SEQ ID NO:35);

5' - CGC TGG GAA ACC TAT TCG G - 3' (SEQ ID NO:36);

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5' - CTG CCA TCC AGT TTC TTC GGG - 3' (SEQ ID NO:37);

5' - GGT GGC ATT GAC AAA TTC TGG - 3' (SEQ ID NO:38);

5' - CCC ACC ATG CGA CAC CAG - 3' (SEQ ID NO:39);

5' - TGT GCA ACG CAA ATG GCA C - 3' (SEQ ID NO:40);

5' - CGA CCC CAA GTT TCC TGT AAG TG - 3' (SEQ ID NO:41);

5' - AGG CAC GAT AGT TGT GGC AGA C - 3' (SEQ ID NO:42);

5' - CAC TCA ACC CAT CCT ACC CAC C - 3' (SEQ ID NO:43); and full-length

complements thereof.

52. (Previously presented) A method for identifying a beta-lactamase in a clinical sample, the method comprising:

providing a pair of oligonucleotide primers specific for nucleic acid characteristic of the OXA family of beta-lactamase enzymes, wherein one primer of the pair is complementary to at least a portion of the beta-lactamase nucleic acid in the sense strand and the other primer of each pair is complementary to at least a portion of the beta-lactamase nucleic acid in the antisense strand;

annealing the primers to the beta-lactamase nucleic acid;

simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product that is complementary to the nucleic acid strands annealed to each primer wherein each extension product after separation from the beta-lactamase nucleic acid serves as a template for the synthesis of an extension product for the other primer of each pair;

separating the amplified products; and

analyzing the separated amplified products for a region characteristic of the beta-lactamase;

wherein the primers are selected from the group consisting of :

5' - CGT CGC TCA CCA TAT CTC CC - 3' (SEQ ID NO:34);

5' - CCT CTC GTG CTT TAG ACC CG - 3' (SEQ ID NO:35);

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5' - CGC TGG GAA ACC TAT TCG G - 3' (SEQ ID NO:36);  
5' - CTG CCA TCC AGT TTC TTC GGG - 3' (SEQ ID NO:37);  
5' - GGT GGC ATT GAC AAA TTC TGG - 3' (SEQ ID NO:38);  
5' - CCC ACC ATG CGA CAC CAG - 3' (SEQ ID NO:39);  
5' - TGT GCA ACG CAA ATG GCA C - 3' (SEQ ID NO:40);  
5' - CGA CCC CAA GTT TCC TGT AAG TG - 3' (SEQ ID NO:41);  
5' - AGG CAC GAT AGT TGT GGC AGA C - 3' (SEQ ID NO:42);  
5' - CAC TCA ACC CAT CCT ACC CAC C - 3' (SEQ ID NO:43); and full-length complements thereof.

53. (Currently amended) A method for identifying a beta-lactamase in a clinical sample, the method comprising:

providing a pair of oligonucleotide primers specific for nucleic acid characteristic of the OXA family of beta-lactamase enzymes, wherein one primer of the pair is complementary to at least a portion of the beta-lactamase nucleic acid in the sense strand and the other primer of each pair is complementary to at least a portion of the beta-lactamase nucleic acid in the antisense strand;

annealing the primers to the beta-lactamase nucleic acid;

simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product that is complementary to the nucleic acid strands annealed to each primer wherein each extension product after separation from the beta-lactamase nucleic acid serves as a template for the synthesis of an extension product for the other primer of each pair;

separating the amplified products; and

analyzing the separated amplified products for a region characteristic of the beta-lactamase;

wherein when the oligonucleotide primers are specific for the OXA family beta-lactamase enzyme designated as OXA-1, the primers are selected from the group of:

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5'- TGT GCA ACG CAA ATG GCA C - 3' (SEQ ID NO:40);

5'- CGA CCC CAA GTT TCC TGT AAG TG - 3' (SEQ ID NO:41); and full-length complements thereof;

[[and]] wherein when the oligonucleotide primers are specific for the OXA family beta-lactamase enzymes designated as OXA-5, 6, 7, 10, 11, 13, or 14, the primers are selected from the group of:

5'- GGT GGC ATT GAC AAA TTC TGG - 3' (SEQ ID NO:38);

5'- CCC ACC ATG CGA CAC CAG - 3' (SEQ ID NO:39); and full-length complements thereof;

wherein when the oligonucleotide primers are specific for the OXA family beta-lactamase enzyme designated as OXA-9, the primers are selected from the group of:

5'- CGT CGC TCA CCA TAT CTC CC - 3' (SEQ ID NO:34);

5'- CCT CTC GTG CTT TAG ACC CG - 3' (SEQ ID NO:35); and full-length complements thereof;

wherein when the oligonucleotide primers are specific for the OXA family beta-lactamase enzyme designated as OXA-12, the primers are selected from the group of:

5'- CGC TGG GAA ACC TAT TCG G - 3' (SEQ ID NO:36);

5'- CTG CCA TCC AGT TTC TTC GGG - 3' (SEQ ID NO:37); and full-length complements thereof;

and wherein when the oligonucleotide primers are specific for the OXA family beta-lactamase enzyme designated as OXA-2, 3, or 15, the primers are selected from the group of:

5'- AGG CAC GAT AGT TGT GGC AGA C - 3' (SEQ ID NO:42);

5'- CAC TCA ACC CAT CCT ACC CAC C - 3' (SEQ ID NO:43); and full-length complements thereof.

54. (New) A method for identifying a beta-lactamase in a clinical sample, the method comprising:

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providing a pair of oligonucleotide primers specific for nucleic acid characteristic of the OXA family of beta-lactamase enzymes, wherein the enzyme is found in a Gram-negative bacterium selected from the group of *Enterbacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, *Providencia spp.*, *Proteus mirabilis*, *Yersinia enterocolitica*, and combinations thereof, wherein one primer of the pair is complementary to at least a portion of the beta-lactamase nucleic acid in the sense strand and the other primer of each pair is complementary to at least a portion of the beta-lactamase nucleic acid in the antisense strand;

annealing the primers to the beta-lactamase nucleic acid;

simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product that is complementary to the nucleic acid strands annealed to each primer wherein each extension product after separation from the beta-lactamase nucleic acid serves as a template for the synthesis of an extension product for the other primer of each pair;

separating the amplified products; and

analyzing the separated amplified products for a region characteristic of a beta-lactamase found in a Gram-negative bacterium selected from the group consisting of *Enterbacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, *Providencia spp.*, *Proteus mirabilis*, *Yersinia enterocolitica*, and combinations thereof.